IN THE SPECIFICATION

Page 1, prior to "Description" add the following:

-- RELATION APPLICATION

This application is a divisional of Serial No. 09/896,032 filed June 29, 2001, which is a divisional of Serial No. 08/892,704, filed September 15, 1997, which is a divisional of Serial No. 08/511,759 filed August 7, 1995. --.

Page 1, paragraph 4, please amend as follows:

EP-A 0 450 931 incorporated by reference discloses the complete nucleotide and amino acid sequence of HCV. In addition a combination of synthetic HCV antigens is described comprising a first HCV antigen from the C domain and at least one further HCV antigen from one ~~of the~~ encoded by non-structural domains NS3, NS4 or NS5 and the envelope domain S. A preferred antigen from the domain NS3 is an antigen denoted C33c which comprises the amino acids 1192 to 1457 of the HCV genome shown in Figure 1 of EP-A-O 450 931.

Page 2, paragraph 3, please amend as follows:

A test for HCV in blood requires a high degree of specificity and sensitivity ~~of the test~~. Furthermore the antigen used for the test should be capable of expression in high yield and- be stable. Previously known antigens from the HCV genome have disadvantages since they do not fulfill one or several of the above requirements.

Page 2, paragraph 4, please amend as follows:

Thus an object of the present invention ~~was~~ is to provide a polypeptide ~~from~~ encoded by the HCV genome in which these disadvantages of the state of the art are at least partially eliminated and which, especially in comparison

with known antigens, has a higher specificity and sensitivity and can be expressed in higher yield and is stable.

Page 3, paragraph 1, please amend as follows:

This object is achieved by a polypeptide which is composed of ~~the~~ amino acids 1207 ± 10 to 1488 ± 10 of a hepatitis C virus and has less than 20, preferably less than 15 foreign amino acids. The polypeptide according to the invention preferably contains the amino acids 1207 ± 5 to 1488 ± 5~~;~~. More preferably they contain amino acids particularly preferably 1207 ± 2 to 1488 ± 2 and most preferably 1207 to 1488 of ~~a~~ hepatitis C virus in which the numbering of the amino acid residues refers to Fig. 1 of EP-A-0 450 931 incorporated by referenced.

Page 3, paragraph 2, please amend as follows:

The polypeptide according to the invention can be derived from an arbitrary HCV isolate, for example from a HCV isolate with a nucleotide sequence as described in EP-A-0 450 931. However, the polypeptide is preferably derived from the HCV isolate from which the clone NS3 described in W092/11370 is derived which was deposited at the "Deutsche Sammlung von Mikroorganismen and Zellkulturen GmbH" (DSM), Mascheroder Weg 1b, 38124 Braunschweig, under the number DSM 6847. The polypeptide according to the invention ~~can be particularly~~ is preferably obtained by recombinant expression of the vector pUC-D26.

Page 3, paragraph 3, please amend as follows:

SEQ ID ~~NO.1~~ NO: 1 shows the amino acid sequence of a polypeptide according to the invention. Amino acids 1 13 are ~~forein~~ foreign amino acids. Amino acids 14 - 295 originate from HCV. The polypeptide according to the invention ~~particularly~~ the preferably contains the amino acids 14 - 295 of the

amino acid sequence shown in SEQ ID ~~NO.1~~ NO: 1 and 2 or an amino acid sequence which is at least 90 % homologous thereto.

Page 4, paragraph 1, please amend as follows:

The present invention also concerns a polypeptide defined as above which contains at least one marker group. All known marker groups come into consideration as the marker group that can be detected in a test system i.e. directly or indirectly detectable marker groups. In this connection a directly detectable marker group is understood as a group which produces a directly detectable signal e.g. a radioactive group, an enzyme group, a luminescence group, a metal complex etc.. On the other hand the marker group can also be an indirectly detectable group e.g. a biotin or hapten group that is detectable by reaction with a suitable binding partner (streptavidin, avidin or anti-hapten antibody) which in turn carries a signal-generating group. The marker group can be coupled in a known manner to the antigen for example via ~~a~~ bifunctional spacer. Such processes for coupling marker groups to peptide antigens are known to a person skilled in the area of immunology and do not need to be described in detail here.

Page 4, paragraph 3, through page 5, please amend as follows:

In addition, the invention concerns a polypeptide as defined above in which one or several of the sulfhydryl groups ~~originating from~~ of cysteine residues are present in ~~a~~ covalently modified form. Examples of suitable covalent modifying groups are maleimidodioxaoctylamine (MADOO); N-methyl-maleinimide (NMM), iodoacetic acid and iodoacetamide. The covalent cysteine modification results in ~~a~~ particularly high specific immunological reactivity.

Page 5, paragraph 1, please amend as follows:

Directly or indirectly detectable marker groups are ~~particularly~~ preferably covalently coupled to the sulfhydryl groups of the polypeptide. Examples of SH-reactive bifunctional linkers for coupling to sulfhydryl groups are maleimidopropylamine (MP), maleimido-ethylamine (MEA) and maleimidodioxaoctylamine (MADOO).

Page 5, paragraph 2, please amend as follows:

In addition, it may be preferable to use a polypeptide according to the invention in which one or several cysteine residues are replaced by other natural or artificial amino acids. Cysteine residues are preferably replaced by structurally analogous α-amino acids e.g. serine or α-aminobutyric acid. Cysteine substitutions lead to ~~a~~ particularly high stability.

Page 5, paragraph 3, through page 6, please amend as follows:

The polypeptide according to the invention has surprising advantages over already known polypeptides. Compared to the antigen C33 described in EP-A-0 450 931 which contains the amino acid residues 1192 to 1457 of the HCV sequence, the polypeptides according to the invention ~~exhibits a~~ exhibits substantially higher specificity which is manifested in a statistically significant lower number of false positive results in negative sera. Compared to the antigen NS3 described in W092/11370 which contains the region of amino acids 1007 to 1534 of the HCV sequence, the polypeptide according to the invention has ~~a~~ considerably higher stability under test conditions. Compared to a polypeptide which contains amino acids 1227 to 1528 from the NS3 region of HCV, the polypeptide according to the invention has the advantage of ~~an~~ improved expression efficiency and a higher sensitivity. Due to these advantages the polypeptides according to the invention ~~is~~ are substantially superior to all previously known HCV antigens from the NS3 region.

Page 6, paragraph 1, please amend as follows:

In addition, the invention concerns ~~a~~ an isolated nucleic acid which codes for a polypeptide according to the invention. A preferred example of such a nucleic acid is a foreign DNA ~~insertion~~ encoding the peptide of interest inserted in the vector pUC-D26.

Page 6, paragraph 2, please amend as follows:

SEQ ID ~~NO.1~~ NO: 1 also shows the nucleotide sequence of a nucleic acid according to the invention which codes for the polypeptides of SEQ ID ~~NO.1~~ NO: 1 and 2. Nucleotides 40 - 885 code for the region of the polypeptide derived from HCV. The nucleic acid according to the invention preferably contains (a) nucleotides 40 - 885 of the nucleotide sequence shown in SEQ ID NO.1 or (b) a nucleotide sequence that corresponds to a sequence from (a) within the scope of the degeneracy of the genetic code.

Page 6, paragraph 3, through page 7, please amend as follows:

The present invention ~~in addition~~ also concerns a vector which contains at least one copy of a nucleic acid according to the invention. The vector according to the invention is preferably a prokaryotic vector i.e. a vector suitable for propagation in a prokaryotic host cell. Examples of such vectors are shown in Sambrook et al, (Molecular Cloning. A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory Press (1989)), especially in chapters 1 to 4 and 17. The vector according to the invention is ~~particularly~~ preferably a circular plasmid. The nucleic acid according to the invention is preferably present on the vector under the control of a promoter sequence which allows expression of the polypeptide according to the invention. A preferred example of a vector according to the invention is pUC-D2 .

Page 7, paragraph 1, please amend as follows:

Furthermore the invention concerns a cell which is transformed with at least one copy of a nucleic acid according to the invention or with a vector according to the invention. The cell is preferably a prokaryotic cell, ~~particularly~~ more preferably a gram-negative prokaryotic cell and most preferably an E. coli cell.

Page 7, paragraph 2, please amend as follows:

The polypeptide according to the invention is preferably used as an antigen in an immunological test procedure. On the other hand the polypeptide can, however, also be used as a helicase protein and, due to its excellent antigenic action, for the production of a vaccine against ~~a~~ HCV infection.

Page 8, paragraph 1, please amend as follows:

The test procedure preferably comprises mixing the sample liquid with a purified labelled antigen P2 and with the purified solid phase antigen P1 in order to obtain a labelled, immobilized complex consisting of labelled antigen, antibody and solid phase-bound antigen. Compared to other test formats for detecting antibodies, the bridge test format leads to an improvement of the sensitivity of the assay, i.e. additional immunoglobulin classes such as IgM are recognized, and of the specificity, i.e., unspecific reactivities with anti-IgG conjugate are reduced.

Page 8, paragraph 2, please amend as follows:

The labelled antigen P2 carries a directly or indirectly detectable marker group as described above. The solid phase antigen P1 can ~~for example be bound~~ be found, for example directly to the solid phase via a bifunctional spacer. However, P1 is preferably a conjugate present in the solid phase of a polypeptide according to the invention and of a reaction partner of a specific

binding system. The other reaction partner of the specific binding system is present bound to the solid phase. Examples of such specific binding systems are biotin/avidin, biotin/streptavidin, biotin/antibiotin, hapten/antihapten, carbohydrate/lectin and antibody or antibody fragment and antibody against this antibody or against the antibody fragment. The antigen P is preferably in the form of biotin conjugate.

Page 9, paragraph 1, please amend as follows:

In such a double antigen bridge test the polypeptide antigen according to the invention is preferably used in a soluble form in order to avoid increases in the blank value and an unfavourable signal/noise ratio due to aggregations of the antigen. For this purpose the antigen is already either expressed in a soluble form in a suitable expression system, or after expression in soluble form, it is renatured in vitro in a known manner. Furthermore in order to avoid the formation of covalently cross-linked molecular aggregates, the immunological test can be carried out under mild reducing conditions (addition o mild reducing reagents, preferably of sulfhydryl reagents, preferably DTT (dithiothreitol) or DTE (~~dithio-erythritol~~ dithioerythritol) in a concentration range of 1 mmol/l to 25 mmol/l) or/and preferably an antigen with covalently modified sulfhydryl groups or/and an antigen with at least partially substituted cysteine residues is used.

Page 9, paragraph 2, please amend as follows:

The polypeptide antigen according to the invention is preferably used in a soluble form in such a double antigen bridge test in order to avoid increases in the blank and an unfavourable signal/noise ratio due to aggregations of the antigen. A detailed description of the bridge test format is given in EP-A-0 280 211. ~~Reference is herewith made to this disclosure.~~ This patent application is incorporated by reference.

Page 9-10, paragraph 3, please amend as follows:

The polypeptide according to the invention can, however, also be used in other test formats. An example of this is an indirect immunoassay for recognizing a specific immunoglobulin by binding to an immobilized specific antigen and indirect detection via a conjugate with a second antibody. In this embodiment of the method according to the invention the sample liquid is incubated with a polypeptide P1 which is (a) bound to a solid phase or (b) is present in a form capable of binding to a solid phase and with a further antibody directed towards P1 which carries a marker group. The antibody to be determined is detected indirectly by determination of the label in the solid phase ~~or/and~~ and/or in the liquid phase, preferably in the solid phase. In this method the signal produced on the solid phase by an immobilized complex of labelled antibody and solid phase-bound antigen is indirectly proportional to the concentration of the antibodies to be determined in the sample liquid.

Page 10, paragraph 1, please amend as follows:

The present invention ~~in addition~~ also concerns a reagent for the immunological determination of an antibody directed towards hepatitis C virus which contains at least one polypeptide according to the invention. If the reagent is used in a double antigen bridge test, it preferably contains at least two polypeptides P1 and P2 wherein polypeptide P1 is (a) bound to a solid phase or (b) is present in a form capable of binding to a solid phase and polypeptide P2 carries a marker group. Binding of the polypeptide P1 to the solid phase can either be achieved by direct binding or by means of a specific binding pair, preferably streptavidin/avidin and biotin. The polypeptide P1 is particularly preferably present in a biotinylated form.

Page 11, paragraph 1, please amend as follows:

A further area of application of the polypeptide according to the invention is to produce vaccines. For this the polypeptides according to the invention are preferably produced in a purified form and then brought into the form of injectable liquids which can either be solutions or suspensions of the polypeptides. The polypeptides can also be enclosed in liposomes. Further constituents of these vaccines ~~are for example~~ may include water, salt solutions, glucose or glycerol. In addition the vaccines may contain small amounts of auxiliary substances such as emulsifiers, buffer substances, and if necessary adjuvants which increase the immune response. The vaccines are usually administered parenterally by injection, preferably subcutaneously or intramuscularly.

Page 13, paragraph, please amend as follows:

A DNA fragment was amplified by means of PCR starting with the clone NS3 (DSM 6847) using primers (1) and (2) whose nucleotide sequence is shown in SEQ ID NO. 3 and SEQ ID NO. 4. Sequences for cloning (BamHI, BspHI, EcoRI restriction cleavage sites) are located at the 5' end of this DNA fragment as well as an ATG codon and an AAA(Lys) codon to increase expression. Restriction sites for HindIII and EcoRI and a stop codon (~~TTA~~TAA) are present at the 3' end. In primers (1) and (2) the region homologous to HCV starts at nucleotide No. 19 .

Page 14, paragraph 1, please amend as follows:

The bacterial pellets from two ~~1-1~~ one liter cultures were resuspended in 200 ml 50 mmol/l Tris-HCl, pH 8.5, 0.2 mg/ml lysozyme and 2 mmol/l dithioerythritol (DTE). Subsequently EDTA (final concentration: 15 mmol/1), phenylmethylsulfonyl fluoride (final concentration: 1 mmol/1) and 4 mg DNase were added. The suspension was mixed for several minutes with a magnetic stirrer and incubated for 45 minutes at 37°C in a water bath.

Page 15, paragraph 1, please amend as follows

Subsequently ~~it~~ this was centrifuged for 20 minutes at 35,000 and °C. The resulting pellet was resuspended in 30 ml 50 mmol/l Tris- C1, pH 8.5, 2 mmol/l DTE, 150 mmol/i DTA and 1.5 % OGP (o tyl-B-D-gluco-pyranoside, Biomol Company). This suspension was stirred vigorously at room temperature with a magnetic stirrer for at least three hours and subsequently centrifuged at 35,000 g and 4°C for 20 minutes.

Page 15, paragraph 2, please amend as follows:

The pellet was dissolved in 100 ml of 8 mol/l urea, 20 mmol/l Tris-HC1, pH 8.5, 2 mmol/1 DTE and stirred. The antigen which was now dissolved, can be frozen at -20°C until further processing.

Page 15, paragraph 3, please amend as follows:

The protein was purified by the chromatographic steps described ~~in the following~~ infra which were carried out at room temperature. The antigen was stored between each of the chromatographic steps at 20°C.

Page 16, paragraph 3, please amend as follows:

The antigen has a size of ca. 41 kDa ~~in the~~ on SDS polyacrylamide gel. The yield was ca. 10 mg antigen per litre culture medium.

Page 18, paragraph 1, please amend as follows:

~~A good~~ Good reactivity is found in all HCV antigen segments with strongly positive sera. In the case of weakly positive NS3-HCV sera the C33 antigen exhibits in some cases the same and in other cases a slightly weaker reactivity in comparison with the antigen according to the invention. However, in the case of D27 a considerably reduced colour reaction with weak NS3-positive sera is found.